



Increment 45/46 Science Symposium

Light Microscopy Module Biophysics -3 (LMM-B3)



Presented by:

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LMM-B3



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Hauptman Woodard Medical
Research Institute, Inc.

NASA Research Announcement (NRA) - 2013 Macromolecular Biophysics

Growth Rate Dispersion as a Predictive Indicator for Biological Crystal Samples Where Quality Can be Improved with Microgravity Growth

- Experiment to be performed in the FIR LMM.
- Test the hypothesis that the presence of growth rate dispersion in macromolecular crystals grown on the ground is an indicator of crystals that can be improved when grown in microgravity.
- Launches scheduled for **February 2016** on SpaceX-10 (MB1), and **2019** on TBD (B1).

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Light Microscopy Module Biophysics -3 (LMM-B3)

- Science Background and Hypothesis
- Investigation goals and objectives
- Measurement approach
- Importance and reason for ISS
- Expected results and how they will advance the field
- Earth benefits/spin-off applications

Science Background and Hypothesis – 1/8

Science Background

- Biological macromolecules make up the machinery, the instruction set, and the scaffold of life. They are smaller than the wavelength of visible light, thus sophisticated techniques are needed to visualize them, and through this process, understand how life works. A structural understanding helps to discern mechanisms; once we understand how the machinery works, we aid, or more commonly impede that machinery through appropriate pharmaceutical design. The principal means to visualize the structure of these macromolecules is X-ray crystallography, a process that measures the diffraction of X-rays from ordered crystals to calculate the atomic structure. The importance of this process is exemplified in over 12 Nobel Prizes awarded to discoveries made from biological structures derived by X-ray crystallography.
- A limiting element to the process is the availability of high-quality, well-diffracting crystals. Quality is defined as a crystal that provides X-ray diffraction data of sufficient completeness and detail (resolution) to see the structure and hence understand the biology of the system. Biological macromolecules often take many days to grow crystals. Growth in reduced acceleration (commonly termed microgravity) on an orbiting spacecraft extends the physical quality of macromolecular crystals through a reduction in the mosaic spread (caused by slight misalignments of the molecules in the crystal lattice and used as a measure of long-range order) and an increase in crystal volume. Resolution (a measure of short-range order) is not directly affected by microgravity, but can still benefit with the correct experimental design to exploit the demonstrated improvements in long-range order. Microgravity growth can yield an improved quality crystal; however, not all samples improve from microgravity growth.
- If we could predict which samples could be improved by crystal growth in microgravity, then the true potential of this medium could be exploited in an efficient manner. Our experiment aims to enable this.

Science Background and Hypothesis – 2/7

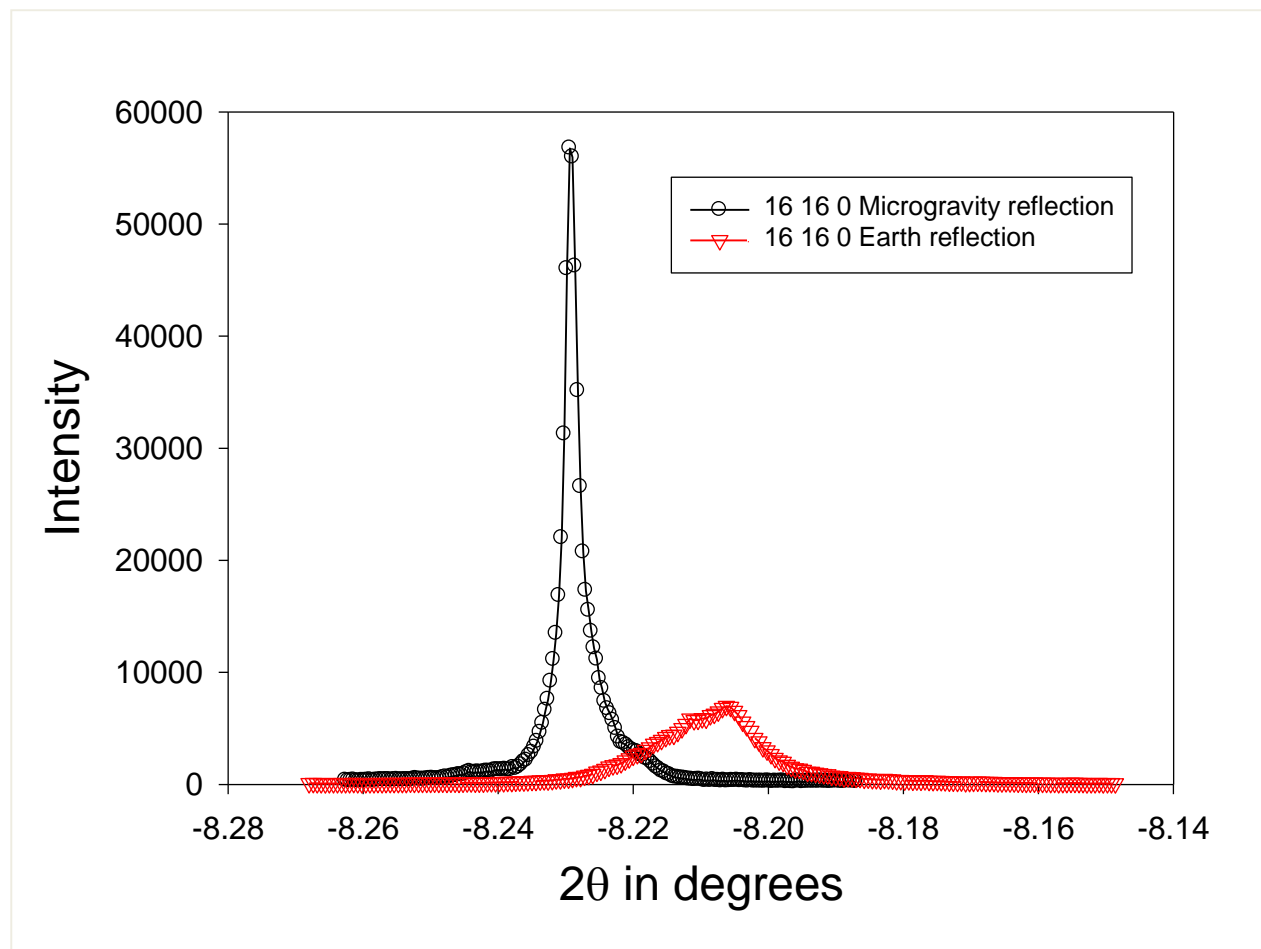
Science Background (cont.)

- Most biological processes, in particular those of health-related interest (both on the ground and in space), occur at the molecular level
- Pharmaceuticals are designed and work at this level
- Biological machinery (proteins, DNA, RNA, viruses, etc.) at this level is smaller than the wavelength of light – we cannot observe them with microscopes

Definitions:

- Mosaicity
 - A crystal can be thought of as an array of domains all slightly misaligned with each other. Perturbations to the misalignment are lumped together into the quantity called mosaicity. A highly mosaic crystal has many imperfections.
- Growth Rate Dispersion
 - Individual crystals starting at the same size, all apparently subjected to identical growth conditions, can grow at different rates
- Quality
 - A high quality crystal has low mosaicity and diffracts strongly to a high resolution

Original experiments investigating microgravity crystal growth (mosaicity)



Identical reflections from microgravity and ground grown lysozyme.

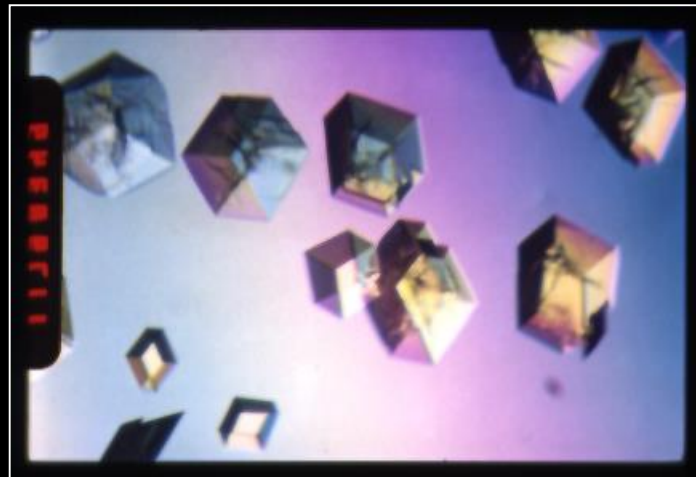
Eight times increase in signal to noise.

The larger illuminated volume only accounted for a doubling.

Microgravity 0.0023 degrees, ground 0.0130 degrees.

Snell et al., Acta Cryst. D51 099-1102 (1995)

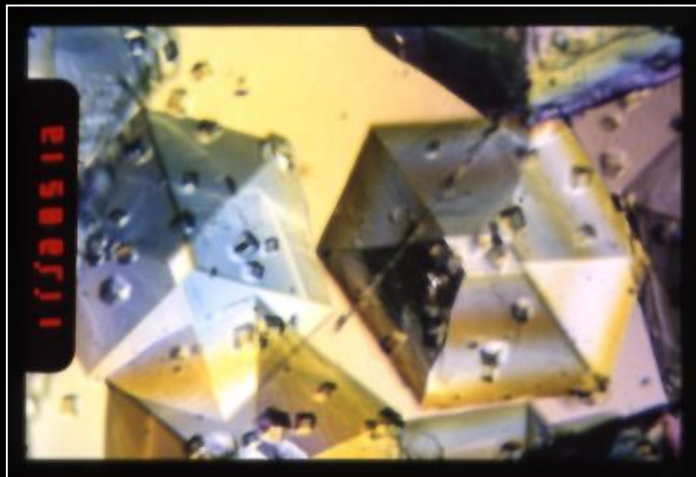
Previous studies on insulin



Images to same scale.

Ground:

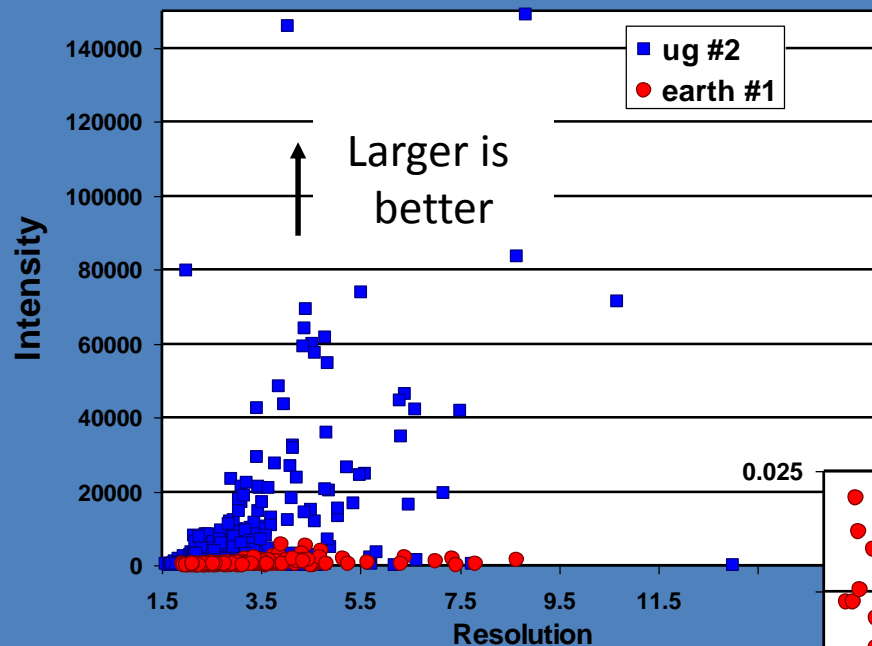
Sedimentation onto the bottom. Clumping of crystals.



Microgravity:

Free floating, unsedimented. had consistently larger diffracting volume > 2 mm in each dimension (34 times larger on average)

From STS-95. Borgstahl, G.E.O., Vahedi-Fardi, A., Lovelace, J., Bellamy, H. & Snell, E.H. Acta Cryst, D57, 1204-1207 (2001).



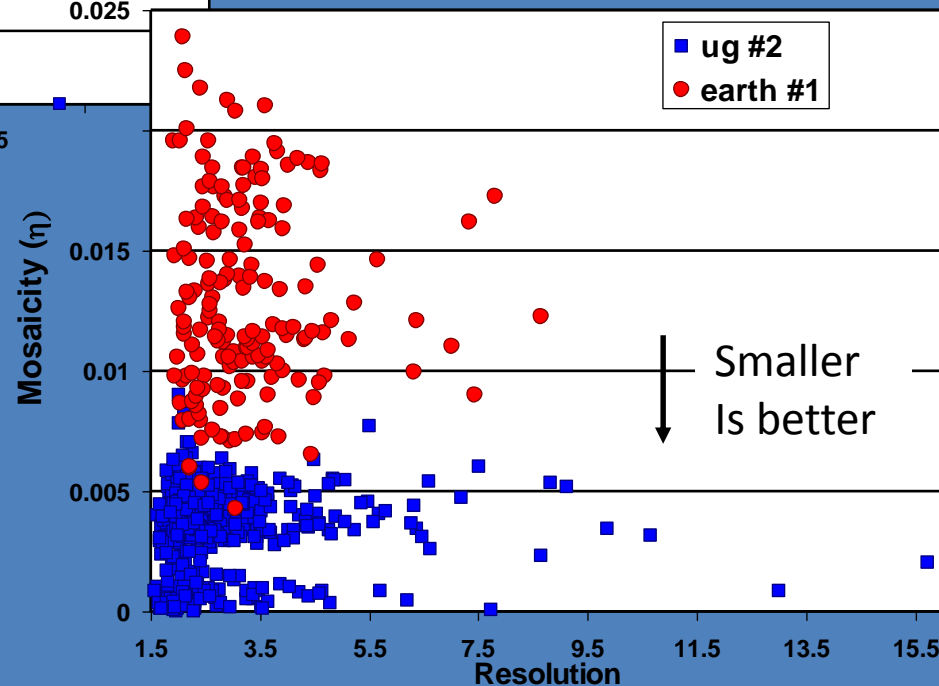
Microgravity Blue

Ground Red

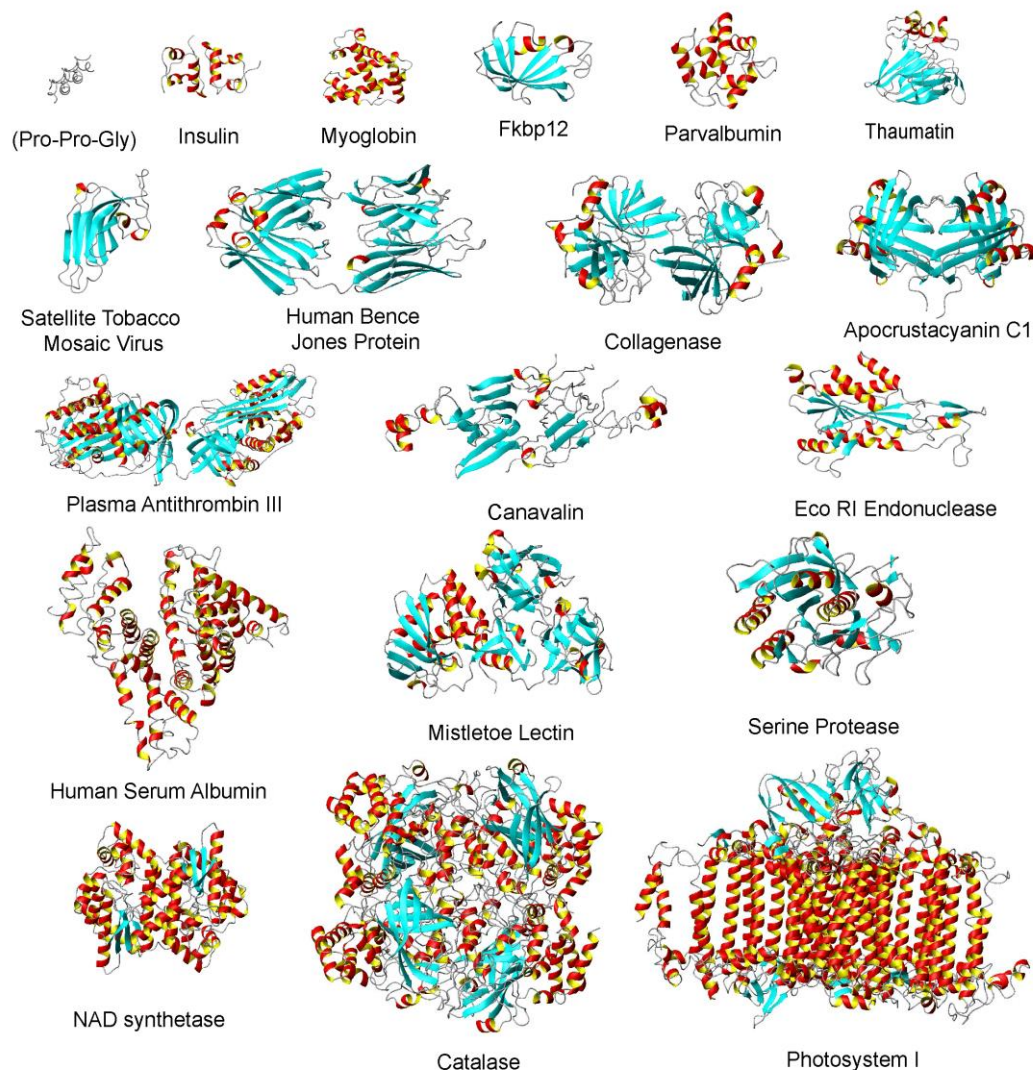
Structural, short range improvement

Physical, long range improvement.

The worst microgravity and best ground crystal data are shown.

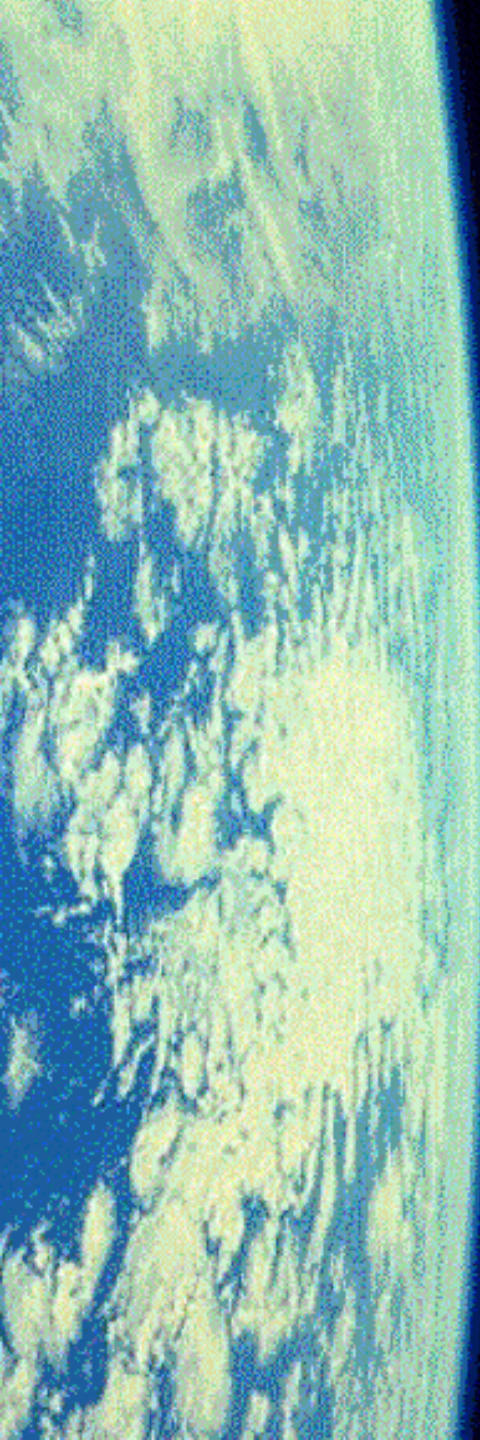


New and Improved Published Macromolecular Structures Resulting from Microgravity Research



References. (Pro-Pro-Gly) - Berisio et al., *Protein Science* (2002), 11, 262-270; Insulin - Smith et al., *Acta Cryst D* (2003) 59, 474-482; Myoglobin - Miele et al., *Acta Cryst.* (2003) D59, 982-988; Fkbp12 - Wilson et al., *Acta Cryst D* (1995) 51, 511-521; Parvalbumin - Declercq et al., *Protein Science* (1999) 8, 2194-2204; Thaumatin - Sauter et al., *Proteins: Struct. Funct. Genet.* (2002) 48, 146-150; Satellite Tobacco Mosaic Virus - Larson et al., *J. Molecular Biology* (1998), 277, 37-59; Human Bence Jones Protein - Terzyan et al., *J. Mol. Recog.* (2003) 16, 83-90; Apocrustacyanin - Habash et al., *Acta Cryst D* (2003), 7, 1117-1123; Plasma Antithrombin III, Skinner et al., *J. Molecular Biology* (1997), 268, 201-209; Canavalin - Ko et al., *Acta Cryst D* (2000), 46, 411-420; Collagenase - Broutin-L'Hermite et al., *Acta Cryst D* (2000) 56, 376-378; Eco RI Endonuclease - Rosenberg et al., structure deposited in PDB, publication in preparation; Human serum albumin - Carter et al., *Science* (1990) 249, 302-303; Mistletoe lectin - Krauspenhaar et al., *Acta Cryst D* (2002) 58, 1704-1707; Serine Protease - Betzel, C. et al., *Biochemistry* (2001) 40, 3080-3088; Catalase - Ko et al., *Acta Cryst D* (1999) 55, 1383-94; NAD Synthetase, Symersky et al., *Acta Cryst D* (2002), 58, 1138-1146; Photosystem I - Klukas et al., *J. Biol. Chem.* (1999), 274, 7351-7360. Not included are the Nucleosome Core Particle - Harp et al., *Acta Cryst D* (2000) 56, 1513-1534; Human interferon-gamma, Ealick et al., *Science* (1991), 252, 698-702; Bacteriophage lysozyme - Evrard et al., *J. Mol. Biol* (1998), 276, 151-164; This poster is not inclusive, it does not include many structures of commercial interest where the coordinates and publication have not been released. Images are not to scale. For reproduction of the poster please contact the author.

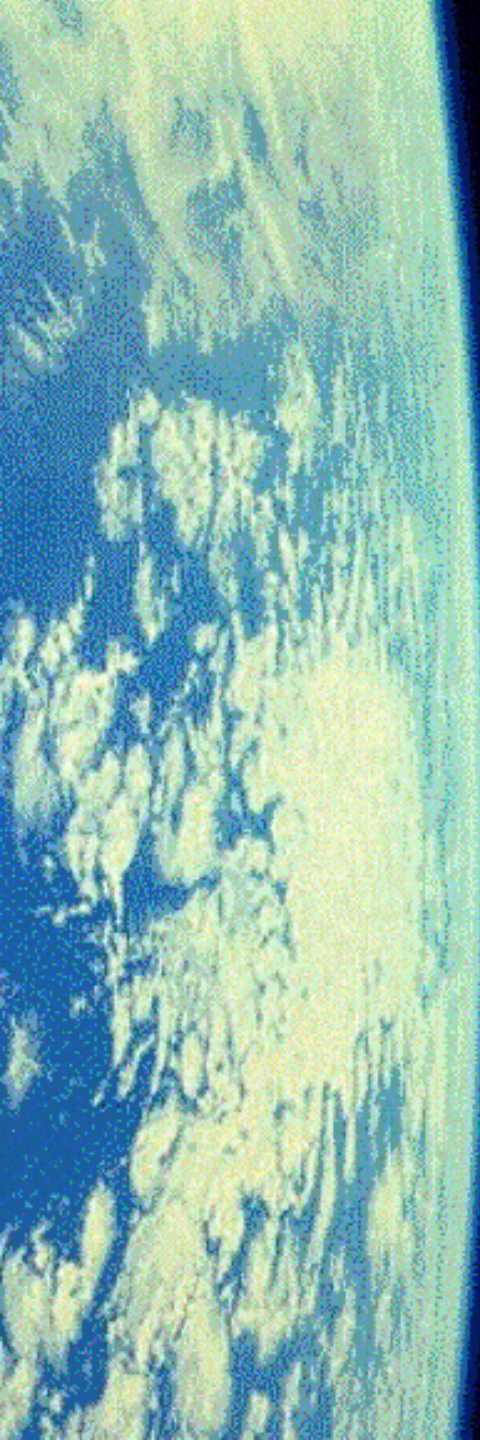
Edward H. Snell, 2003.



Relationship between growth rate dispersion and mosaicity

Small molecule studies have shown a direct relationship between mosaicity and growth rate dispersion.

- Sherwood and Ristic (2001) see reduced mosaicity with reduced growth rate dispersion for sodium chlorate, potash alum and sodium nitrate.
- The same effect is also seen for sodium chloride (Cunningham *et al.*, 1991) and ammonium sulfate (Meadhra *et al.*, 1995).
- Larger molecules such as sucrose (Berglund *et al.*, 1984) and fructose (Johns *et al.*, 1990) show dispersion.
- Growth rate dispersion studies for macromolecules have been limited. Ovalbumin (Judge *et al.*, 1995) and lysozyme (Cherdrungsi, 1999) are two example cases.

- 
- In small molecule studies X-ray data shows that growth rate dispersion is related to crystal mosaicity. The greater the growth rate dispersion the greater the mosaicity.
 - Mosaicity dramatically improves in microgravity grown crystals.
 - Microgravity crystals with reduced mosaicity can be used to increase the data signal-to-noise and hence resolution.

Hypothesis

- Growth rate dispersion is a predictive experimental technique for improvement in microgravity. Crystals benefiting most from microgravity will be those that show most growth rate dispersion on the ground.

LMM-B3 Investigation Goals and Objectives

Goals:

- Monitor growth rate dispersion of crystals grown on the ground and in microgravity, to determine if there is a correlation between the physical qualities of the resulting crystals with those measurements.
- Use molecular biology techniques to alter the crystallization contacts and shift the growth rate dispersion properties of a single protein from low to high to test our predictive hypothesis.
- Extend the study to a selection of good and poorly diffracting crystals on the ground and confirm that those displaying high-growth rate dispersion on the ground are those that are improved on orbit and generate improved structural data when their quality is exploited.

LMM-B3 Investigation Goals and Objectives

Objectives:

1. Produce closely related protein constructs that display different growth rate dispersion. Measure the 'attractiveness' of each construct for modeling the inter-particle forces, diffusion and convection.
2. Measure and compare growth rate dispersion on the ground and then in microgravity.
3. Characterize the quality of both ground and microgravity grown crystals. Sophisticated X-ray analysis along with X-ray structural data collection will be used to characterize the resulting crystals.
4. Link the knowledge about inter-particle forces, measured acceleration levels, calculated flow rates and the resultant X-ray analysis to develop tests that can be carried out on the ground to predict those samples that would be improved in microgravity.

LMM-B3 Measurement approach

We will be using a flight-hardened Commercial-Off-The-Shelf (COTS) microscope

[pictured on next page]

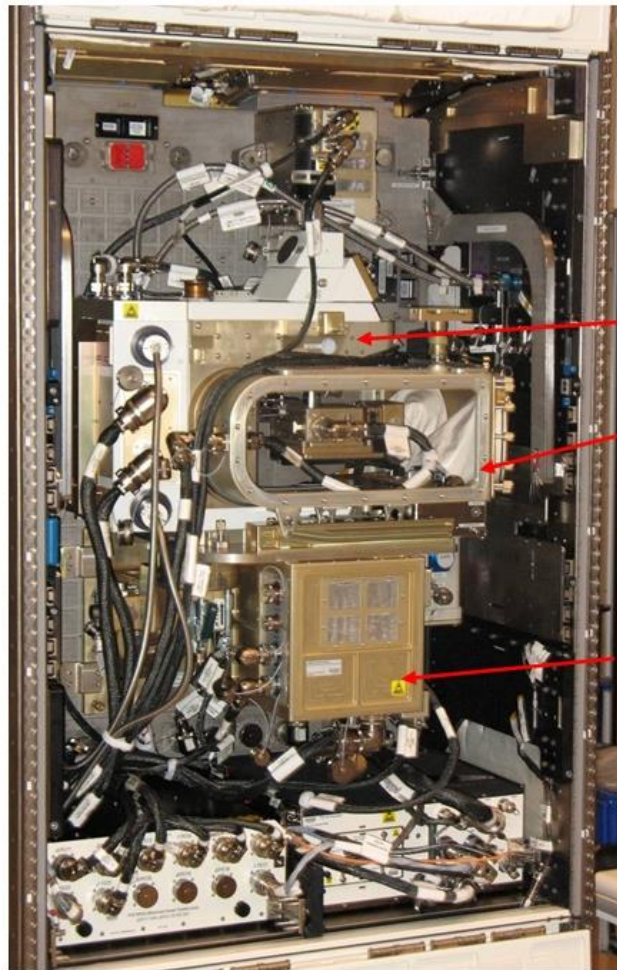
and a

Macromolecular Biophysics sample module

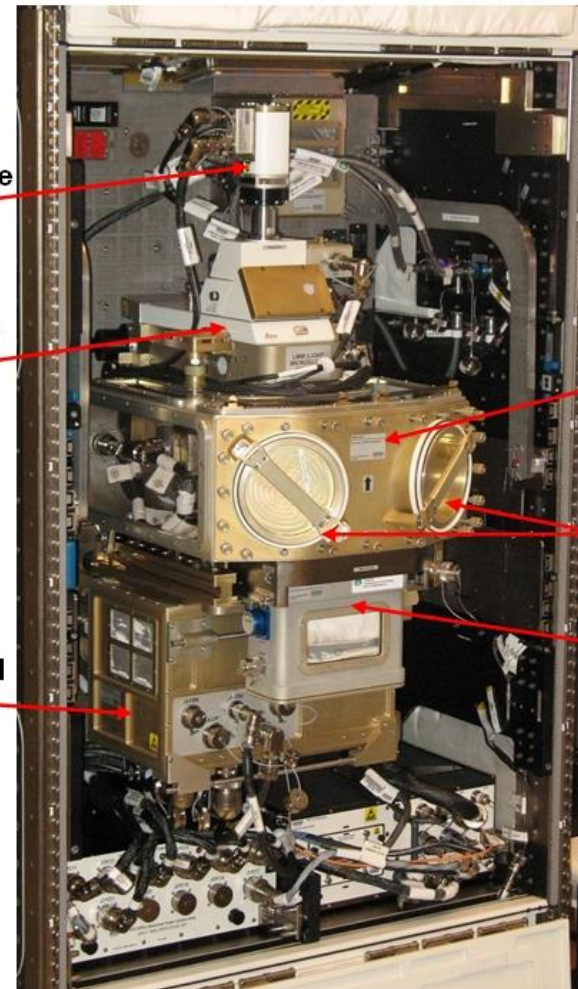
[pictured later]

Measurement approach – 1/5

Light Microscopy Module (LMM) in the Fluid Integrated Rack (FIR)



LMM in the Closed Position or Operating Configuration



LMM in the Open Position or Installation/Service Configuration

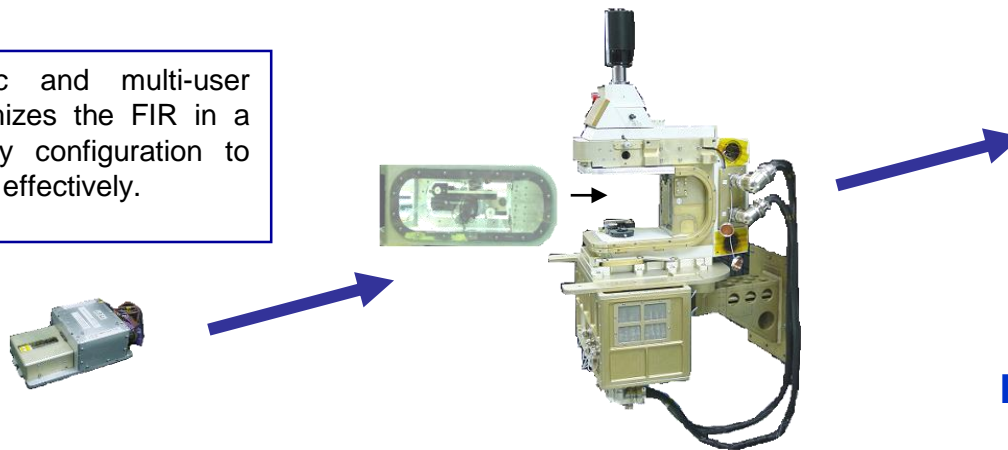
Measurement approach – 2/5

LMM Implementation Philosophy

Philosophy: Maximize the scientific results by utilizing the existing LMM capabilities. Develop small sample modules and image them within the LMM

Payload specific and multi-user hardware customizes the FIR in a unique laboratory configuration to perform research effectively.

Light Microscopy Module



Payload Specific Hardware

- Sample Cell with universal Sample Tray
- Specific Diagnostics
- Specific Imaging
- Fluid Containment

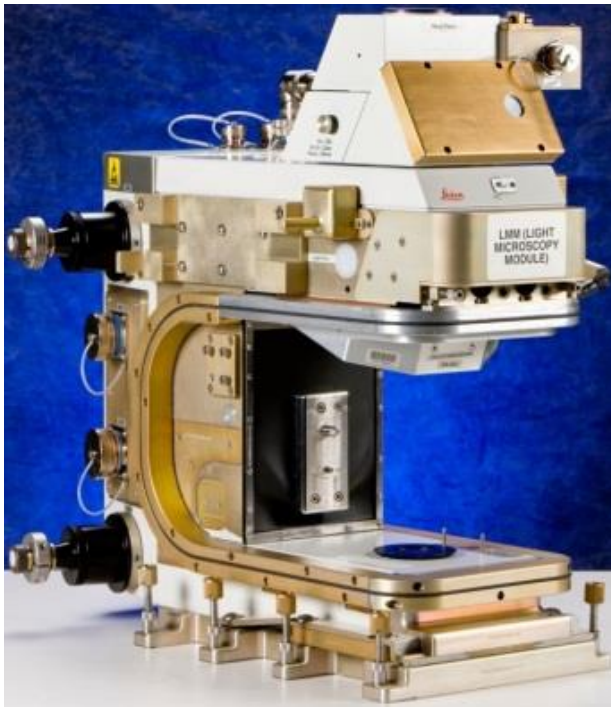
Multi-Use Payload Apparatus

- Test Specific Module
- Infrastructure that uniquely meets the needs of PI experiments
- Unique Diagnostics
- Specialized Imaging
- Fluid Containment

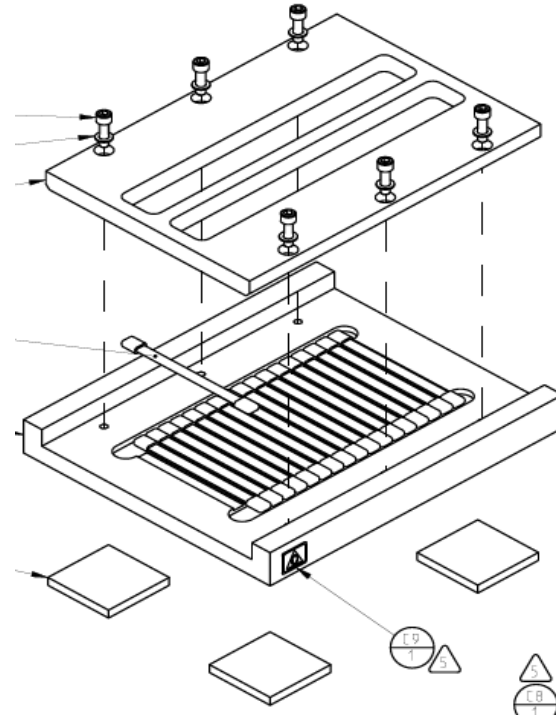
FCF Fluids Integrated Rack

- Power Supply
- Avionics/Control
- Common Illumination
- PI Integration Optics Bench
- Imaging and Frame Capture
- Diagnostics
- Environmental Control
- Data Processing/Storage
- Light Containment
- Active Rack Isolation System (ARIS)

LMM-B3 Measurement approach – 3/5



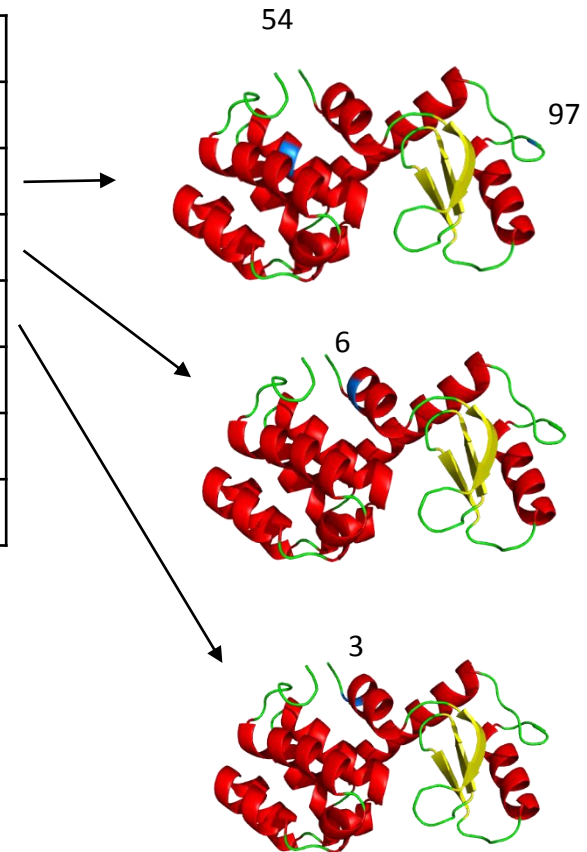
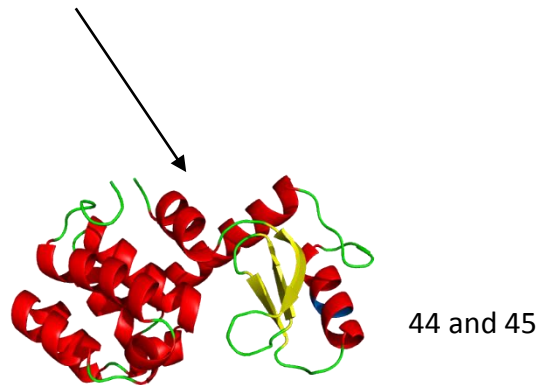
Light Microscopy Module
(LMM)



LMM-B3 Sample Assembly
drawing will contain up to 16
square 50 mm capillaries - Snell

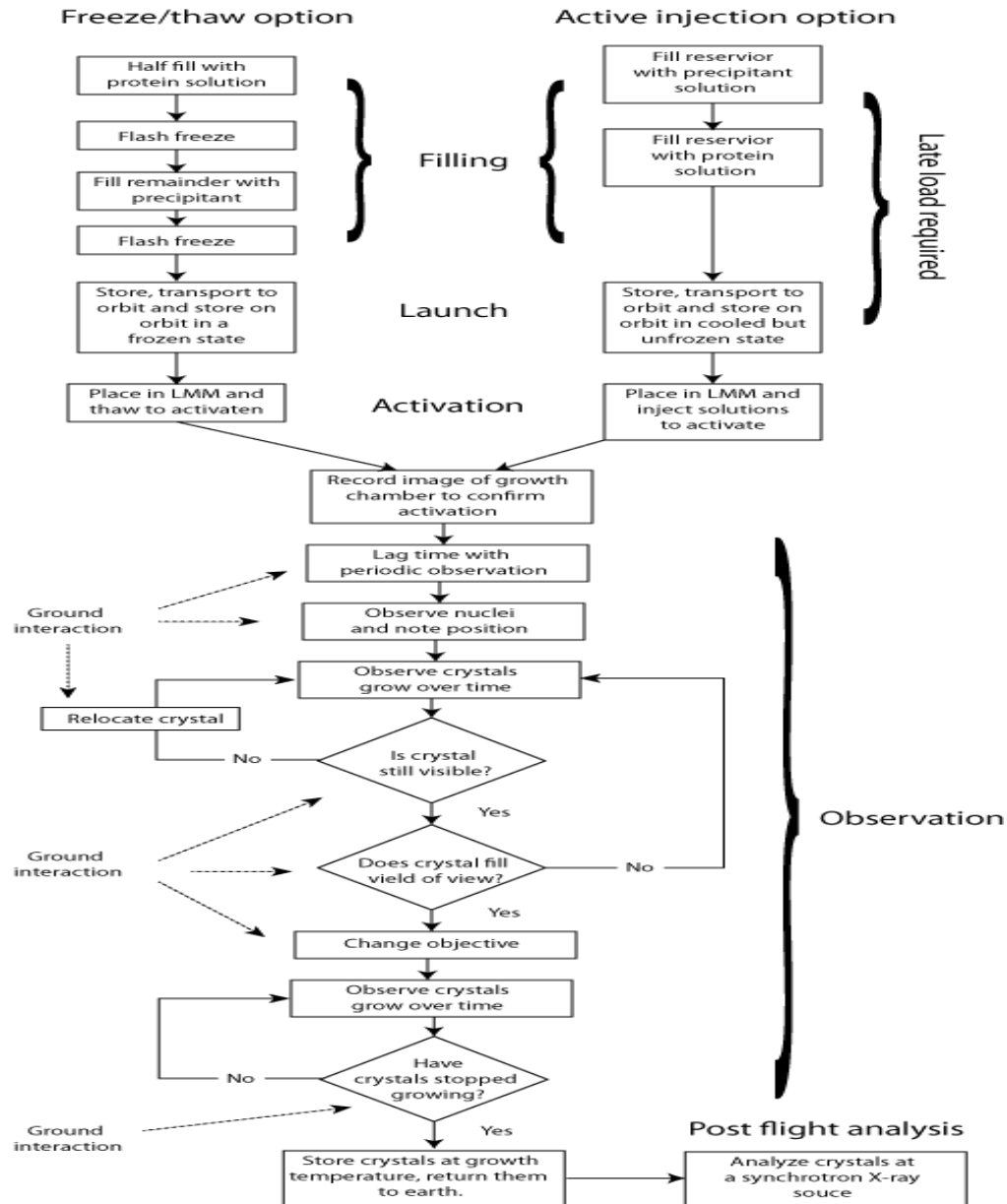
Constructs in the laboratory

#	Construct	Yield per 6l of Broth
1	T4 lys Wildtype	150 mg
2	T4 lys WT* (C54T, C97A)	~20 mg
3	T4 lys M6I	88 mg
4	T4 lys I3P	40 mg
5	T4 Lys S44E WT*	85 mg
6	T4 Lys S44F WT*	53 mg
7	T4 lys E45A WT*	54 mg



(Produce closely related protein constructs that display different growth rate dispersion. Measure the 'attractiveness' of each construct for modeling the inter-particle forces, diffusion and convection).

LMM-B3 Flow Chart of ISS Experiment



- Capsules dropping to earth after being lifted by balloon offer $10^{-2}g$ to $10^{-5}g$ over approx. 1 min. Sounding and sub-orbital rockets offer longer, $10^{-5}g$ periods, e.g. 7 min in the case of Consort which reaches a height of 300km or 15 min in the case of MASER which reaches a height of 900km.
- There are sudden negative acceleration forces upon return to earth and parachute recovery, and sounding rockets do not achieve the necessary microgravity time for the completion of our experiments.
- The nucleation and growth of macromolecular crystal samples is a slow process that takes place over a period of days. As convective flows are thought to negatively affect macromolecule crystal growth, an environment that will allow the formation of purely diffusive transport over a period of days to weeks is therefore sought.
- Ramachandran et al. (1995) developed numerical models for flow and transport under different g-levels. They determined that the classical solution to the vertically heated flat plate could be used to describe the velocity and mass transport in the vicinity of a macromolecule crystal. This gave a Sherwood number (the ratio of total mass flux transport to that under diffusive conditions) for protein crystal growth conditions of 1.0 at $10^{-5}g$, i.e. at this level the transport is diffusion limited.

The International Space Station is currently the only platforms capable of providing the time and g-level environments required for macromolecule crystal growth experiments.

Expected results and how they will advance the field

Long-duration protein crystal growth experiments on the ISS with photo documentation, and subsequent analysis of the comparisons between 1g and μ g crystals, will enable a more complete understanding of why proteins and other macromolecules often form more perfect crystals in microgravity than they do on earth

If hypothesized results are proven, tests can be developed to carry out on the ground to predict those samples that could be improved by growth in microgravity

Earth benefits / spin-off applications

- If it could be predicted which macromolecular samples could be improved by crystal growth in microgravity, then the true potential of this medium could be exploited in an efficient manner
- Structural biology of protein-protein complexes and integral membrane proteins are currently a high NIH priority due to their importance for systems biology, disease mechanisms and structure-guided drug development.
- Hauptman Woodward Medical Research Institute has a data archive of millions of time-resolved crystallization images (from over 14,000 different biological macromolecules) that could be used to identify candidates likely to benefit from crystallization in microgravity.



LMM-B1

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BACKUP SLIDES

Mission Success Criteria for LMM-B1 (DeLucas)

Success Level	Accomplishment
Minimum Success	<ul style="list-style-type: none">(1) In the first mission, crystallization of one or more crystals of a minimum of two proteins displaying different growth rate dispersion on the ground.(2) Imaging data at a minimum of twenty time points capturing and recording initial growth.(3) Images that capture both the start and cessation of growth.(4) Successful extraction of at least six crystals from each successful sample and subsequent X-ray data collection.
Significant Success	<ul style="list-style-type: none">(1) Crystallization of multiple crystals of more than two proteins displaying different growth rate dispersion on the ground.(2) Imaging data at a minimum of forty time points(3) Images that capture both the start and cessation of growth and capture a full range of different crystal sizes.(4) Successful extraction of tens of crystals from all samples and subsequent X-ray data collection.
Complete Success	<ul style="list-style-type: none">(1) Crystallization of multiple crystals of all samples flown.(2) Capture of at least 75% of the planned imaging data.(3) X-ray characterization of at least six crystals from each sample.

Experimental control

- Initial observations will be controlled from the ground to locate the initial crystal positions.
- If a crystal is discovered to have moved out of frame, ground controlled observations will be repeated to reacquire the crystal location.
- Ability to move microscope position and focus to capture z slices of images across the whole depth of the cell (dependent on optics depth of field).
- Ability to accomplish this remotely (i.e. not at a NASA site) – crystallization experiments once the lag phase has past can take several days.

Environmental control and monitoring

- Acceleration data will be recorded within the LMM to identify deviations of at least 10 micro-g Residual Acceleration over frequencies of at least 0.1 Hz to 10 Hz. If the LMM is hard attached to the rest of the ISS calculations of the acceleration at the LMM based on recording elsewhere are acceptable.
- Temperature will be maintained at the sample chambers under a specific temperature condition ranging from 12-24°C at +/- 1°C, the desired temperature TBD.
- Temperature data will be recorded at or close to the sample chambers at a minimum of 10 minute intervals with an accuracy of 0.1°C
- The experiment should be conducted during a period that minimizes low frequency g-jitter, i.e. avoiding planned reboosts or docking.

Post experiment storage

- Sample cells will be removed from the microscope and stored at the growth temperature at controlled or insulated conditions such that they remain within $\pm 1^{\circ}\text{C}$ of that temperature (TBD). Note, this could be the actual ISS temperature.
- Sample cells will be returned to earth while maintained within $\pm 1^{\circ}\text{C}$ of the growth temperature. They are not to be frozen after the experiment, this destroys the crystals.

In flight data

Images of the growth chamber and crystals are required during the experiment.

The following data are required:

- After experiment activation (filling or thawing) initial images of each sample chamber are required.
- During the location of initial crystals, real time or close to real time imaging is required to confirm crystal location.
- Images of the growing crystals are required but a subsection of those images at a sample rate TBD is permissible.
- Information on the microscope position to go with the images is required.
- A time stamp with each image received is required.

Post flight samples

Sample cells are to be returned to the ground for crystal extraction and the cells must allow extraction.

The following data and samples are required

- Timeliness: within 30 seconds of real-time during real-time operation mode to correlate accelerometer data and visual observations with onboard activities.
- Acceleration data over the mission duration covering deviations of at least 10 micro-g Residual Acceleration over frequencies of at least 0.1 Hz to 10 Hz.
- Temperature data during storage, observations, post observation and return to earth.
- Sample cells returned to the ground for crystal extraction
- A complete set of high-resolution images, identified by sample cell, objective, microscope position and time stamp when recorded.